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# Age-Related Changes of Plasma Alkaline Phosphatase and Inorganic Phosphorus, and Late Ossification of the Cranial Roof in the Spanish Imperial Eagle (*Aquila adalberti* C. L. Brehm, 1861)

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## ABSTRACT

Plasma alkaline phosphatase and inorganic phosphorus levels were determined for 52 nestling Spanish imperial eagles from two wild populations and 22 captive adults and subadults (10 adults and 12 subadults). The exact age was known for all birds. Mean alkaline phosphatase and inorganic phosphorus were higher in chicks than in the captive adults and subadults. Sex differences were not observed, and nestlings from different populations showed similar values. No significant regression described the relationship between age and alkaline phosphatase or inorganic phosphorus throughout the nestling period. However, alkaline phosphatase and inorganic phosphorus decreased significantly throughout the subadult period, with age explaining 98.2% and 50.5% of the variation in alkaline phosphatase and inorganic phosphorus levels, respectively. Non-fully-ossified zones were measured in frontal bones of another 12 subadult eagles that died at known ages. Ossification increased throughout the subadult period and was significantly correlated with expected levels of alkaline phosphatase or inorganic phosphorus (i.e., values predicted from the regression equations derived from the first analysis). Minimum alkaline phosphatase levels and full ossification of the cranial roof coincided with puberty onset. We conclude that, in subadult Spanish imperial eagles, decreasing alkaline phosphatase and inorganic phosphorus values are related to the ossification of frontal bones, although a contribution of other unknown processes of late ossification cannot be excluded, and alkaline phosphatase (but not inorganic phosphorus) may be a useful parameter for age-predicting purposes.

## Introduction

The orthophosphoric monoester phosphohydrolase, or alkaline phosphatase, is a zinc-containing enzyme widely distributed in the body, though mainly associated with tissues that have either a high cellular turnover, as the intestinal epithelium, or a high anabolic rate, as bone-forming osteoblasts (Bell 1971). Studies in wild bird species have shown that mean plasma alkaline phosphatase activity is higher in immature birds than in adults (Puerta et al. 1989, 1992; Costa et al. 1993; Polo et al. 1994). Also, nestlings of red kites (*Milvus milvus*) and black kites (*Milvus migrans*) (Viñuela et al. 1991), bald eagles (*Haliaeetus leucocephalus*) (Redig 1991), and white spoonbills (*Platalea leucorodia*) (de le Court et al. 1995) show alkaline phosphatase and phosphate values significantly higher than those in corresponding subadults and adults. Although not all alkaline phosphatase activity in the bird plasma originates in osteoblasts (Bell 1971), these variations are believed to result from the different rate of osteoblastic activity and bone growth in young and adults (Viñuela et al. 1991; Puerta et al. 1992; Costa et al. 1993; Polo et al. 1994). However, few attempts have been made to correlate normal variations in plasma alkaline phosphatase or phosphate with morphometrical measurements suggestive of bone formation. In birds of prey, those relationships are likely to occur during the nestling period, when full development of long bones is attained (see, e.g., Viñuela et al. 1991). Once birds have fulfilled their somatic growth, full ossification of some patches of the skeleton such as the cranial roof may be delayed (Ferrer 1993), but it is unknown whether these local processes of late ossification affect alkaline phosphatase and/or phosphate levels.

In addition, changes in plasma alkaline phosphatase and phosphate during development could be used to estimate the age of wild birds, but this potential utility has not been investigated. In the studies mentioned above, the exact age for each specimen was probably unknown, for they have been grouped into two (Puerta et al. 1989, 1992; Polo et al. 1994; de le Court et al. 1995) or four major age classes at most (Costa et al. 1993). Therefore, individual changes over time could not be examined, and appropriate regression equations based on age could not be derived. Other bird studies, in which the age-related changes in alkaline phosphatase (and phosphate) were investigated on an individual basis, are limited to the first days posthatching (Hoffman et al. 1985) or to a part of the nestling period (Viñuela et al. 1991).

The Spanish imperial eagle (*Aquila adalberti*) is the most endangered bird of prey in Europe (Ferrer 1993). Conservation projects intended to follow the population dynamics started early in the 1970s, and a number of specimens have been routinely banded as chicks ever since. The later entry of some of those banded eagles into recuperation centers and their successful long-term maintenance there have provided an opportunity to examine a variety of plasma parameters in animals whose exact age is known (P. M. Dobado-Berrios and M. Ferrer, unpublished data). We report here on individual plasma values of both alkaline phosphatase and inorganic phosphorus ( $P_i$ ) from a wide range of ages (the 74 birds analyzed independently for such purposes were between 34 d and 18 yr old). There were two main aims to the present study: first, we attempted to find a correlation between alkaline phosphatase or  $P_i$  levels and ossification of the cranial roof in subadult Spanish imperial eagles, and second, we determined whether alkaline phosphatase and  $P_i$  values could be reliable indicators of age in this species.

## Material and Methods

### Plasma Chemistry

**Animals.** From 1986 to 1989 a total of 52 nestling (age, 34–75 d), 12 subadult (90 d to 5 yr), and 10 adult (5–18 yr) Spanish imperial eagles (*Aquila adalberti*) were bled for plasma analyses. Subadults and adults will be referred to as “(sub)-adults” when both groups are considered as a whole. The exact age (in days) was known for all the birds. The age of the chicks was determined by former checks of the nests, and all (sub)adults were previously banded as nestlings in their nests. All the chicks lived in the wild, with 41 individuals from a population in southwestern Spain (Parque Nacional de Doñana, ca. 37° N, 6°5′ W) and 11 individuals from central Spain (ca. 40°29′ N, 4°28′ W). These groups, whose mean ages were not statistically different ( $51 \pm 1$  and  $55 \pm 3$  d, respectively), will be referred to as population 1 and population 2, respectively. (Sub)adult eagles belonged to the Raptor Recuperation Centers of Estación Biológica de Doñana (Matalascañas) and Instituto para la Conservación de la Naturaleza (Sevilleja de la Jara) and were examined macroscopically and radiographed there. Birds with clinical signs of disease or traumatic injuries that could affect the data were not used. Healthy (sub)adults were confined in pens, exposed to ambient outdoor temperatures and natural photoperiods, and fed fresh whole rabbit and chicken carcasses ad lib. Sex distinction was made through a discriminate analysis based on the measurements of the tarsus and forearm (Ferrer 1993). The chick group consisted of 23 males and 29 females (18 males and 23 females from population 1 and five males and six females from population 2), and the (sub)adult group consisted of 11 males and 11 females.

The differences in the mean age (in days) between sexes were not statistically significant for any of the studied age groups.

**Blood Collecting Procedures and Biochemical Analyses.** Blood samples of up to 2 mL were taken with a heparinized syringe from the brachial vein, which was carefully pressed after bleeding to prevent hematoma occurrence. All samples were collected between 1100 and 1500 hours to minimize any variation in blood chemistries caused by the birds’ daily rhythm (Ferrer [1990] and references therein). Blood was carefully placed into tubes containing lithium heparin to prevent coagulation and formation of bubbles. The blood collection tubes were coated with ice, stored in insulated containers, and carried to the laboratory (Valme Hospital, Seville) within 8 h after blood withdrawal. Then each blood sample was centrifuged at 3,000 rpm for 10 min, and plasma was stored at  $-60^\circ\text{C}$  until analysis. Biochemical analyses were performed on a computer process-controlled multichannel autoanalyzer (Hitachi 747, Tokyo) with commercial kits (Boehringer-Mannheim Biochemica, Mannheim). Plasma activity of alkaline phosphatase ( $\text{U L}^{-1}$ ) was determined by the paranitrophenyl-phosphate method, and the  $P_i$  ( $\text{mg dL}^{-1}$ ) was measured by the molybdenum blue reaction.

### Ossification of the Cranial Roof

For ossification measurements, we studied 12 skulls from subadult eagles that died at known ages. A light source was put into the encephalic cavity by passing a flexible lamp through the foramen occipitale magnum. Each non-fully-ossified zone in the pars frontalis of the frontal bone was thus revealed as a translucent surface (Fig. 1) and was traced on an onionskin sheet and then measured with a digital planimeter (Tamaya, Tokyo). Cranial length and the distance between postorbital processes were also measured for each skull (Fig. 1).

### Statistical Analyses

All data are expressed as mean  $\pm$  standard error (SE), and ranges are also provided. Statistical analyses were carried out with the software package SIGMASTAT 1.02 (Jandel Scientific, Corte Madera, Calif.). Differences were considered significant at  $P < 0.05$ . Before comparing two independent groups, data were tested for normality with the Kolmogorov-Smirnov test (with Lilliefors’s correction) and for equal variance with the Levene median test. If the data groups passed both tests, the comparison was made by independent Student’s *t*-test. If either the normality or equal variance assumption was violated, the comparison was made by the Mann-Whitney rank-sum test. In order to analyze some paired measurements regarding the ossification of subadult eagles, the Wilcoxon signed-rank test was used. A two-way ANOVA was used to compare many independent data groups if the previous normality and equal

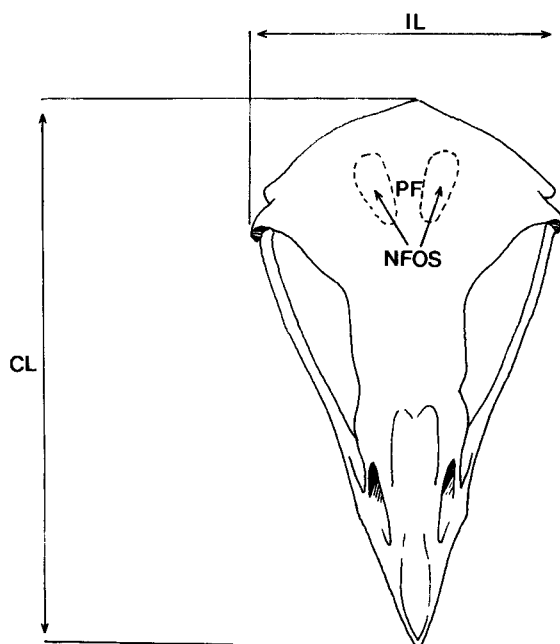


Figure 1. A representation of the eagle skull showing the location of the non-fully-ossified zones (NFOS) in the pars frontalis (PF) of the frontal bone. CL, cranial length; IL, interorbital length.

variance tests had been passed. Pearson's product-moment correlation was used to examine the linear concomitant variation of two variables if they met normality and homoscedasticity assumptions. If they did not, the Spearman rank-order correlation was used. The best-fitting equations to describe the relationships between two variables were explored by simple linear and polynomial regression analyses. When appropriate, log and inverse transformations of the dependent and/or the independent variable were performed. Only those regression analyses that met normality and homoscedasticity assumptions were accepted.

## Results

### Biochemical Analysis

(Sub)adult alkaline phosphatase values were in general agreement with those previously reported for captive Spanish imperial eagles (*Aquila adalberti*) aged 4–20 yr (Polo et al. 1992), but  $P_i$  levels were lower in our study. This may have been due to differences in assay procedures, since our data were within the range of those values obtained from captive (sub)adult kites (*Milvus*) in a study in which the molybdenum blue reaction was also used (Viñuela et al. 1991). Alkaline phosphatase and  $P_i$  values have not been previously reported for nestling Spanish imperial eagles.

Both alkaline phosphatase activity and  $P_i$  concentration were higher in the chick (alkaline phosphatase,  $1,295 \pm 47$  U L<sup>-1</sup>,

range, 484–1,973;  $P_i$ ,  $6.7 \pm 0.3$  mg dL<sup>-1</sup>, range, 4.2–14.8;  $n = 52$  for both chemistries) than in the (sub)adult eagle plasma (alkaline phosphatase,  $64 \pm 28$  U L<sup>-1</sup>, range, 3–487,  $n = 20$ ;  $P_i$ ,  $3.2 \pm 0.3$  mg dL<sup>-1</sup>, range, 1.6–7.9,  $n = 21$ ) (Mann-Whitney rank-sum test,  $P < 0.001$  for all comparisons). There was no statistical difference between sexes. Spearman's relational statistics indicated that age and alkaline phosphatase and age and  $P_i$  were negatively correlated, whereas alkaline phosphatase (AP) and  $P_i$  values described positive concomitant variations. Those relationships were found both for males ( $r_{\text{age,AP}} = -0.609$ ,  $P < 0.005$ ,  $n = 32$ ;  $r_{\text{age,P}_i} = -0.518$ ,  $P < 0.002$ ,  $n = 33$ ;  $r_{\text{AP,P}_i} = 0.414$ ,  $P = 0.021$ ,  $n = 31$ ) and females ( $r_{\text{age,AP}} = -0.600$ ,  $P < 0.005$ ;  $r_{\text{age,P}_i} = -0.662$ ,  $P < 0.005$ ;  $r_{\text{AP,P}_i} = 0.621$ ,  $P = 0.005$ ;  $n = 40$  for all correlations).

### Nestling Alkaline Phosphatase and $P_i$

The mean alkaline phosphatase values recorded were  $1,277 \pm 54$  U L<sup>-1</sup> (range, 845–1,973,  $n = 41$ ) for population 1 and  $1,357 \pm 104$  U L<sup>-1</sup> (range, 484–1,846,  $n = 11$ ) for population 2. A two-way ANOVA showed that these differences were not statistically significant ( $P = 0.496$ ), that differences between sexes were not significant ( $P = 0.943$ ), and that there was not a significant interaction between sex and population ( $P = 0.831$ ). Similarly, differences in  $P_i$  levels between population 1 ( $6.9 \pm 0.4$  mg dL<sup>-1</sup>, range, 4.2–14.8,  $n = 41$ ) and population 2 ( $5.7 \pm 0.2$  mg dL<sup>-1</sup>, range, 4.5–6.3,  $n = 11$ ) were not statistically significant (Mann-Whitney rank-sum test,  $P = 0.303$ ). In addition, we did not find any significant linear or nonlinear regression for assessing the relationships between age and alkaline phosphatase activities (Fig. 2A) or between age and  $P_i$  concentrations (Fig. 2B), and alkaline phosphatase and  $P_i$  variables did not correlate throughout the nestling period. Those results were found for both the whole sample of chick data and the partial samples of data grouped according to sex and/or population.

### (Sub)adult Alkaline Phosphatase and $P_i$

The relatively low sample size forced us to group (sub)adult males and females together for the regression analyses based on age. A significant age-related decrease in both alkaline phosphatase (Fig. 3A) and  $P_i$  values (Fig. 3B) was found throughout the subadult period, with the respective regressions being improved by inverse transforming the age data (measured in days). The forms of the regression equations are

$$\text{alkaline phosphatase} = -7.94 + 43,560(\text{age}^{-1}) \quad (1)$$

( $R^2 = 0.982$ ,  $F = 989.8$ ,  $P < 0.001$ ,  $n = 20$ ), and

$$P_i = 2.465 + 529.921(\text{age}^{-1}) \quad (2)$$

( $R^2 = 0.505$ ,  $F = 19.4$ ,  $P < 0.001$ ,  $n = 21$ ).

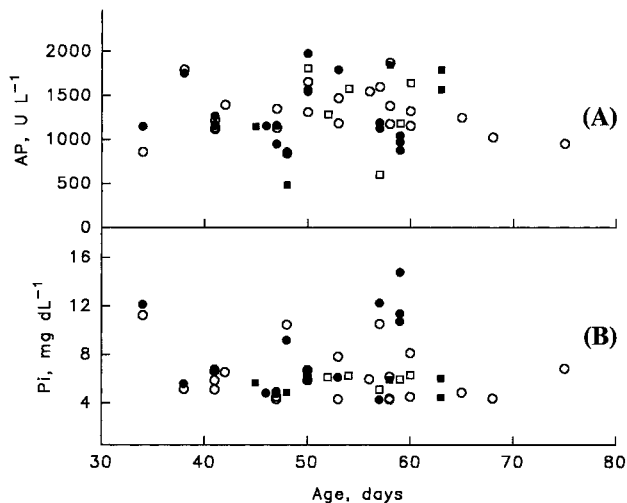


Figure 2. A, Plasma alkaline phosphatase (AP) of 52 nestling eagles; B,  $P_i$  values of the same eagles. The symbols are as follows: filled circles, males from population 1; open circles, females from population 1; filled squares, males from population 2; and open squares, females from population 2. No significant regression described the relationship between age and alkaline phosphatase or  $P_i$  throughout the nestling period.

Raw residuals from the regressions of alkaline phosphatase and  $P_i$  based on the age were generated. Student's *t*-tests showed that differences in the mean residuals between males and females were not statistically significant ( $P = 0.557$  for alkaline phosphatase residual comparison and  $P = 0.385$  for  $P_i$  residual comparison). Alkaline phosphatase activities and  $P_i$

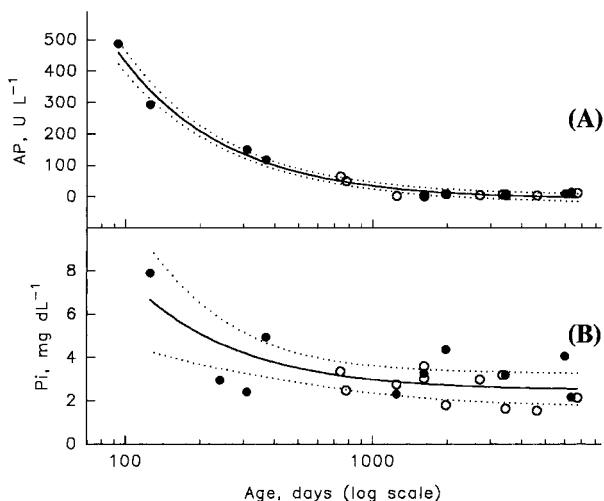


Figure 3. Age-related decrease in plasma values of (A) alkaline phosphatase (AP) and (B)  $P_i$  throughout the (sub)adult eagle period. The solid lines are the best-fitting regressions of each chemical on age, as obtained by pooling from all males (filled circles) and females (open circles). The dotted lines are the 99% confidence limits for the corresponding regressions.

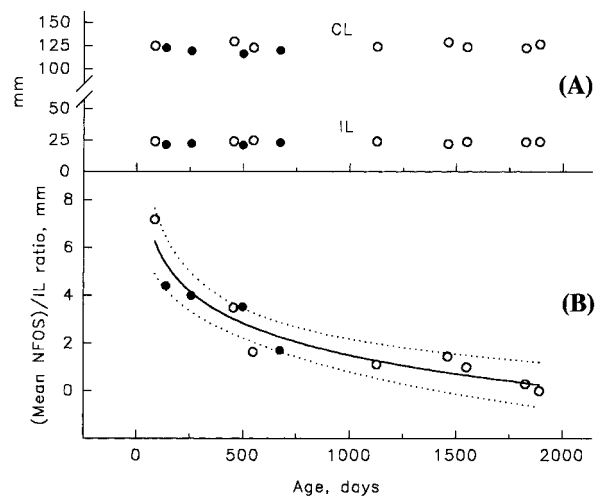


Figure 4. A, Cranial length (CL) and interorbital length (IL) of four subadult male (filled circles) and eight subadult female eagles (open circles) that died at known ages. B, Ossification of the cranial roof. The solid line is the best-fitting regression of the mean non-fully-ossified surface in the frontal bone (NFOS, corrected for IL values) on age, as obtained by pooling data from all males and females. The dotted lines are the 99% confidence limits for the regression.

levels described positive concomitant variations throughout the whole (sub)adult period ( $r = 0.724$ ,  $P < 0.001$ ,  $n = 19$ ), in spite of the fact that  $P_i$  reached a minimum before alkaline phosphatase did (the latter occurring approximately at age 1,800–2,000 d).

#### Ossification of the Cranial Roof in the Subadult Eagles: Predicted Relationships to Plasma Alkaline Phosphatase and $P_i$ Values

Both cranial length and interorbital length remained invariable at different ages throughout the subadult period (Fig. 4A), but the mean values were significantly greater in the females (cranial length =  $125.77 \pm 0.94$  mm, range, 123.00–129.79; interorbital length =  $24.02 \pm 0.29$  mm, range, 22.25–25.05;  $n = 8$ ) than in the males (cranial length =  $119.75 \pm 1.32$  mm, range, 116.46–122.87; interorbital length =  $22.04 \pm 0.43$  mm, range, 21.25–23.08;  $n = 4$ ;  $P < 0.005$  for all comparisons).

One left and one right non-fully-ossified surface was always present in each skull. Both surfaces were of similar size for each subadult eagle, as assessed by comparing paired left and right maximum diameters (Wilcoxon signed-rank test,  $P = 0.700$ ) and paired left and right minimum diameters ( $P = 0.689$ ). This allowed us to use a mean value for each pair of non-fully-ossified surfaces. Mean values ranged from  $175.212 \text{ mm}^2$  (mean maximum diameter = 20.63 mm, mean minimum diameter = 10.075 mm) for a 84-d-old fledgling to  $7.1676 \text{ mm}^2$  (mean maximum diameter = 3.635 mm, mean



minimum diameter = 1.905 mm) for a 1,825-d-old eagle. The oldest subadult bird whose exact age was known (1,890 d) showed full ossification of its frontal bones. Ossification was also full in all the skulls from other seven eagles with complete adult plumage (> 5 yr old) (data not shown). The relationship between mean non-fully-ossified surface and age (in days) throughout the subadult period could be described by the following equation: mean non-fully-ossified surface =  $20.67 + 13,020(\text{age}^{-1})$  ( $R^2 = 0.844$ ,  $F = 54.2$ ,  $P < 0.001$ ,  $n = 12$ ).

In order to improve that regression, mean non-fully-ossified surface values were standardized according to the sex-dependent cranial length and interorbital length measurements. Thus, ratios of mean non-fully-ossified surface to cranial length and ratios of mean non-fully-ossified surface to interorbital length (in millimeters) were calculated, and the relationships between each of those variables and age were explored. The best-fitting regression equation was accomplished by interorbital length standardization and log transforming the age data (in days) (Fig. 4B): mean non-fully-ossified surface/interorbital length =  $14.84 - 4.448 \times \log(\text{age})$  ( $R^2 = 0.906$ ,  $F = 96.5$ ,  $P < 0.001$ ,  $n = 12$ ).

Mean raw residuals from the regression of mean non-fully-ossified surface (corrected for interorbital length) on the log-transformed age were similar in males and females ( $t$ -test,  $P = 0.375$ ).

Because of the impossibility of simultaneously obtaining plasma samples and cranial measurements from the same specimens, the expected values of alkaline phosphatase and  $P_i$  for the birds that provided the ossification data were predicted by using regression equations (1) (Fig. 3A) and (2) (Fig. 3B). Thus, the correlation between the true ossification values and the expected alkaline phosphatase or  $P_i$  data for the same subadult eagles could be explored. The results are shown in Figure 5. Log transformation of the chemical variables was used to convert the regressions to linear form. The mean non-fully-ossified surface (corrected for interorbital length) and the predicted alkaline phosphatase activity were positively correlated (Fig. 5A;  $r = 0.948$ ,  $P < 0.001$ ,  $n = 12$ ), as were the mean non-fully-ossified surface (corrected for interorbital length) and the predicted  $P_i$  concentration (Fig. 5B;  $r = 0.940$ ,  $P < 0.001$ ,  $n = 12$ ). Mean raw residuals from the regression of the expected log-transformed alkaline phosphatase and  $P_i$  values on the mean non-fully-ossified surface (corrected for interorbital length) were similar in males and females ( $t$ -test,  $P = 0.147$  for alkaline phosphatase residual comparison and  $P = 0.896$  for  $P_i$  residual comparison). The relationship between degrees of ossification and alkaline phosphatase activity enabled differentiation between subadult eagles by age (Fig. 5A), with the full ossification of the frontal bone and the minimum plasma alkaline phosphatase values being reached at the same age (1,890 d). Also, the birds were classified correctly according to the relationship between degrees of ossification and  $P_i$  levels (Fig. 5B), but age discrimination was poor within the range

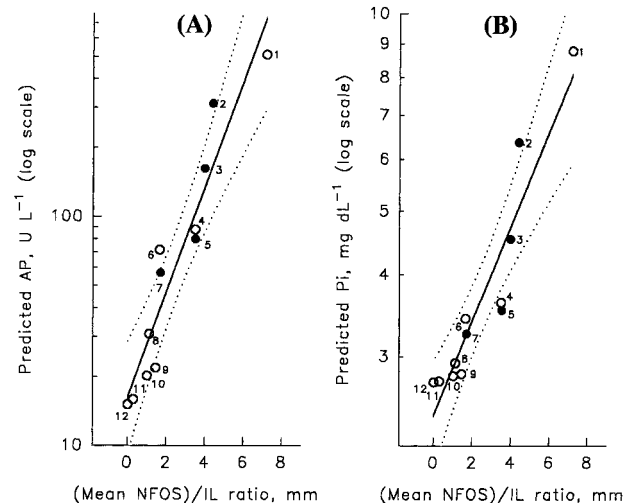


Figure 5. Relationship between (A) plasma alkaline phosphatase (AP) or (B)  $P_i$  and ossification of the cranial roof throughout the subadult eagle period. Chemical values were estimated from the regression equations derived from our previous analysis (Fig. 3). The solid lines are the best-fitting regressions of predicted values of alkaline phosphatase or  $P_i$  on true values of mean non-fully-ossified surface in the frontal bone (NFOS, corrected for interorbital length [IL]), as obtained by pooling data for all four males (filled circles) and eight females (open circles). The dotted lines are the 99% confidence limits for the corresponding regressions. Numeric labels for the symbols represent the exact age (in days) for each specimen: (1) 84; (2) 136; (3) 256; (4) 455; (5) 498; (6) 547; (7) 673; (8) 1,125; (9) 1,460; (10) 1,549; (11) 1,825; and (12) 1,890.

1,460–1,890 d, when  $P_i$  concentration is at a minimum before the ossification of the cranial roof is completed.

## Discussion

We provide strong evidence that supports the hypothesis that decreasing plasma levels of both alkaline phosphatase and  $P_i$  throughout the subadult period of the Spanish imperial eagle (*Aquila adalberti*) are related to the gradual ossification of the frontal bone in this species. Contribution of other patches of delayed ossification to those biochemical changes cannot be completely excluded, although it seems unlikely. Long bones of imperial eagles do not undergo further longitudinal growth after the nestling period (M. Ferrer, personal observation), as is also the case with other raptorial species (Viñuela et al. 1991), and radiological examination of normal subadults reveals the cranial roof as the only area in the skeleton with visible signs of incomplete ossification (M. Ferrer, personal observation).

Virtual stabilization of alkaline phosphatase activity at minimum values by the fifth year coincided with the full ossification of the cranial roof. It is interesting that this is also the age at which most Spanish imperial eagles replace their juvenile brown plumage by the definitive black one and become repro-

ductively mature (Ferrer 1993). It is well known that gonadal growth is photoperiodically induced in birds (Wingfield and Kenagy 1991), but eyes are not the only means for photoreception. In some species, extraretinal encephalic receptors are involved directly in the photo-induced gonadotropic stimulation (Benoit 1964; Menaker and Keatts 1968; Underwood and Menaker 1970; Menaker 1971; Epple and Stetson 1980), with the response varying with the amount of light that penetrates through the top of the head (Menaker et al. 1970). Sites for extraretinal photoreception include hypothalamic areas (Benoit 1964; Yokoyama et al. 1978; Epple and Stetson 1980; van Tienhoven 1981) and cells of the pineal gland (Deguchi 1979; Epple and Stetson 1980), an evagination of the caudal diencephalic roof that extends dorsally toward the skull (Sturkie 1986). Some cytological changes observed in the avian pineal seem to coincide with the beginning of sexual function (Ralph 1970), and a role for the gland in the achievement of sexual maturity of the Japanese quail (*Coturnix japonica*) has been suggested (Saylor and Wolfson 1967). Little attention has been paid to pineal function in control of body color change in birds, but avian pineal homogenates have a powerful effect on the skin pigmentation of amphibians (e.g., the tadpole bioassay in Epple and Stetson [1980]). The full ossification of the eagle cranial roof may cause changes in the lighting conditions perceived by the pineal and/or other extraretinal receptors and may be involved in the endocrine events associated with puberty onset and acquisition of adult plumage.

We failed to find significant variations in either alkaline phosphatase activities or  $P_i$  concentrations throughout the nestling period of the eagles. This is in agreement with results reported for growing emus (*Dromaius novaehollandiae*) (Costa et al. 1993). However, increases in alkaline phosphatase levels in the first week posthatching have been described in American kestrels (*Falco sparverius*) (Hoffman et al. 1985), and alkaline phosphatase and  $P_i$  values of chick red kites (*Milvus milvus*) and black kites (*Milvus migrans*) could be fitted to quadratic curves that were at a maximum at age 34–38 d, though the percentage of variation that was accounted for by the relationship between alkaline phosphatase or  $P_i$  and age represented 28% at most (Viñuela et al. 1991). We cannot exclude an initial rise in the eagle alkaline phosphatase and/or  $P_i$  levels prior to the formation of a plateau at least at age 34 d, that is, the age of the youngest nestling analyzed in our study.

Age explained virtually all of the variation in the plasma alkaline phosphatase values throughout the subadult period (98.2%) but only half of the variation in the  $P_i$  concentrations (50.5%). The changes that we describe are reminiscent of those reported by Takenaka et al. (1988) in an age profile of Japanese macaques (*Macaca fuscata*). In vertebrates, phosphorus is clearly involved in a number of physiological or biochemical processes other than osteogenesis (Fowler 1986), which can explain the lack of a close relationship between plasma  $P_i$  levels and age. Therefore, only the plasma alkaline phosphatase assay,

if properly used, may be a reliable age-predicting test for the Spanish imperial eagle. Its application should exclude specimens with fracture repair, specific metabolic bone diseases, hepatic damage, and aspergillosis because of their abnormally elevated alkaline phosphatase values (Fowler 1986). Moreover, accurate estimations should be obtained only within the interval from fledging to age 5 yr. The predictions would be unreliable in older birds, since their alkaline phosphatase levels remain rather stabilized at a minimum value. However, the proposed range covers the whole immature period in the species, when eagles exhibit a quite uniform brown plumage and their age can be hardly ascertained according to morphological criteria (Ferrer 1993).

The usefulness of the plasma alkaline phosphatase measurements as indicators of the age of subadult Spanish imperial eagles profits from the following features. First, the relationship between enzyme activity and age can be described by a linear equation; that is, unlike the quadratic and higher-degree polynomial forms (see Viñuela et al. 1991), only one value of age can be predicted for each given value of alkaline phosphatase. Second, enzyme activity is not sex dependent, which agrees with previous studies on some wild nonbreeding birds (Puerta et al. 1989; Peinado et al. 1992) and mammals (see, e.g., Seal et al. 1975). Breeding avian females could, however, show high plasma alkaline phosphatase levels related to eggshell formation (Bell 1971; Sturkie 1986; de le Court et al. 1995), but egg laying is quite a rare event in Spanish imperial eagles younger than 5 years old (but see Ferrer 1993). Third, data on chicks suggest no geographic effect on eagle alkaline phosphatase measurements, as is also true of mammals (Seal et al. 1975). This fact is important for a wide application of the alkaline phosphatase test, since a number of other blood chemistries and enzyme activities, which are influenced by the qualitative and quantitative composition of the diet (see, e.g., Ferrer [1990] and references therein) and the presence of environmental toxicants in food material (see, e.g., Hoffman et al. 1985), can differ among the local wild populations of a bird species (P. M. Dobado-Berrios and M. Ferrer, unpublished data). Finally, other preliminary data indicate that alkaline phosphatase values do not show daily rhythms in birds (M. Ferrer, unpublished data). Further studies are needed to confirm the reliability of the plasma alkaline phosphatase assay for predicting age in other bird species. Apart from providing fine estimations throughout the subadult period, alkaline phosphatase values could discriminate between conspecific immature and mature individuals that have a similar appearance.

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